5. DISCUSSION

Soil salinity disturbs the plant–microbe interaction, which is a critical ecological factor to help further plant growth in degraded ecosystems (Paul and Nair, 2008). The use of specific microbe antagonists which stimulate plant growth and are natural enemies of pathogens allows a considerable decrease in the use of agrochemicals which are now being used for plant growth stimulation and control of diseases (Lugtenberg et al., 2001). Development of such a stress-tolerant microbial strain associated with the roots of agronomic crops can lead to improved fertility of salt-affected soils (Egamberdieva and Kucharova, 2009).

The use of beneficial microbes in agricultural production systems started about 60 years ago. The effect of plant growth-promoting bacteria on the growth and nutrient uptake of various agricultural crops was well addressed by Kloeper et al. (1980), Lifshitz et al. (1987), Kloeper and Beauchamp (1992), Okon et al. (1998), Lugtenberg et al. (2001) and Glick et al. (2007). Biological control of plant disease by rhizobacteria was also previously reported by other authors (Adesemoye et al., 2008). There is now increasing evidence that the use of beneficial microbes can enhance plants’ resistance to adverse environmental stresses, viz., drought, salts, nutrient deficiency, and heavy metal contaminations (Glick et al., 2007).

The genus *Azospirillum* is widely distributed in the rhizosphere of tropical and subtropical grasses (Bashan and Holguin, 1997). The mechanisms by which *Azospirillum* sp. can exert a positive effect on plant growth was probably composed of multiple effects including synthesis of phytohormones, N₂-fixation, nitrate reductase activity and enhancing mineral uptake (El-Komy et al., 2003).
In the present research, bacterial population was recorded more in the collected soil samples followed by fungal population and the actinobacteria population in the soil samples were very low. The high incidence of *Azospirillum* in tropical soils may be attributed to the low level of available nitrogen or to higher temperature requirements of these organisms (Favilli *et al.*, 1984; Florenzano and Favilli, 1984). The occurrence of community population of *Azospirillum* was also estimated in the present study. The highest *Azospirillum* population of $16.33 \times 10^4$ CFU/g of soil sample was recorded Pudhuchattiram location followed by $15.35 \times 10^4$ CFU/g of soil sample (Ponnanthittu), $15.00 \times 10^4$ CFU/g of soil sample (Manampadi), whereas the lowest population of $1.89 \times 10^4$ CFU/g of soil sample was observed in Poondiyankuppam location. Scott *et al.* (1979) reported the predominance of nif strains of *Azospirillum lipoferum* and *Azospirillum brasilense* in the rhizosphere of rice.

Smith *et al.* (1984), Albrecht *et al.* (1989) and Harris *et al.* (1989) reported the low *Azospirillum* population in soil. O’Hara *et al.* (1981) suggested the occurrence of *Azospirillum* population within the range of 0.001 – 1.0 percent of total bacterial population. Neyra *et al.* (1995) proposed the use of “EPS mediated flocculated cell forms of *Azospirillum*” as a delivery system for the enhancement of growth and yield of crop plants under stress conditions including, soil salinity. The occurrence of *Azospirillum*, as PGPR, in the rhizosphere of agricultural field soil has been reported by many authors (Ashraf *et al.*, 2004; Nadeem *et al.*, 2006; Gholami *et al.*, 2009; Puente *et al.*, 2009). The results of the present study revealed the predominance of *Azospirillum* in tomato rhizosphere soils in the coastal areas of Cuddalore District, Tamil Nadu, India.

All the isolates obtained from tomato rhizosphere soils of coastal soils of Cuddalore district were subjected to different characters *viz.*, growth
in NFB, BMS agar, Gram’s reaction, motility and presence of PHB. The isolates of Azospirillum were further subjected to various biochemical tests. Azospirillum brasilense did not produce acid from glucose, biotin requirement, nitrate reductase activity is negative. These parameters were used to differentiate A. brasilense from A. lipoferum. Biochemical characterization studies clearly revealed that the Azospirillum isolates viz., AZ - 1, AZ - 2, AZ - 4, AZ - 6, AZ - 7, AZ - 9, AZ -13, AZ -14, AZ -16, AZ -18, AZ -19 and AZ - 20 were identified as A. brasilense. The isolates viz., AZ - 3, AZ - 5, AZ - 8, AZ - 10, AZ – 11, AZ - 12, AZ – 15 and AZ - 17 were identified as A. lipoferum. Occurrence of Azospirillum in maize soils has been reported by many workers (Balandreau et al., 1997; Baldani and Dobereiner, 1980). The results of the present study also clearly revealed the occurrence of Azospirillum in the rhizosphere soils of tomato grown at coastal areas of Cuddalore District in Tamil Nadu, India.

Soil contains myriad’s of populations of soil microorganisms, which interact with each other in soil and also with crop plants in soil nutrient mobilization. Among nutrients required by the plant, nitrogen and phosphorus are most amenable for microbial transformation and also make them available to plants at a time when crop demands. Nitrogen fixation is unique processes which makes its availability to crop plants by prokaryotic group of microorganisms which reduce the atmospheric nitrogen to ammonia and then to amino acid and protein (Subba Rao, 1999).

The in vitro nitrogenase activity and cell nitrogen content of Azospirillum isolates was investigated. Among the twenty isolates identified, AZ - 8 obtained from rhizosphere soil of tomato recorded the maximum nitrogenase activity of 496.00 n moles of C₂H₄ produced mg⁻¹ of protein h⁻¹ and cell nitrogen of 49.00 mg g⁻¹ of cell weight. The minimum nitrogenase activity of 160.24 n moles of C₂H₄ mg⁻¹ of protein h⁻¹ and cell nitrogen
content of 15.20 mg g⁻¹ was recorded in AZ - 20. Hurek et al. (1988) reported that in the rhizosphere of Kallar grass, the strains of *Azospirillum lipoferum* fixed more nitrogen than *Azospirillum brasilense* as evidenced by their nitrogenase activity. In the present study also, the maximum nitrogen fixation was recorded by the *Azospirillum lipoferum* when compared to *Azospirillum brasilense*.

Siripin *et al.* (2000) screened 35 isolates of N₂-fixing bacteria to assess the production of plant growth promoting substances. Each strain had different potential in N₂-fixing ability and had difference in physiology and morphology of the colonies and the cells. N₂-fixing bacterial inoculation increased vetiver growth and development.

The indole acetic acid (IAA) and gibberellic acid (GA₃) producing potential of *Azospirillum* isolates obtained from the rhizosphere of tomato were investigated. All the twenty isolates of *Azospirillum* produced IAA and GA₃ and the quantity ranged from 22.16 to 88.72 µg 25 ml⁻¹ broth for IAA and 3.13 to 9.56 µg 25 ml⁻¹ broth for GA₃. The *Azospirillum* isolate AZ -8 Pudhuchattiram location produced the maximum amount of 88.72 µg of IAA 25ml⁻¹ and 9.56 µg of GA₃ 25 ml⁻¹ of Nitrogen free malate broth. The *Azospirillum* isolate AZ - 20 Poondiyankuppam location produced the minimum amount of 22.16 µg IAA 25 ml⁻¹ of the culture broth and 3.13 µg GA₃ 25 ml⁻¹ of the culture broth. Patiyuth *et al.* (2000) revealed that the N₂-fixing bacteria (*Azospirillum*) produced plant growth hormone, Indole-3-acetic acid (IAA) at 30-40 µg/ml in the broth media. *Azospirillum* grew well outside and inside the vetiver root.

Bacterial siderophores (Pseudobactin and ferrioxamine B) were inefficient as iron sources for plants and the rhizosphere siderophore producing bacteria can be in competition with the plant for iron. In fact, the vast majority of research on microbial siderophores in the rhizosphere is
associated with their biocontrol activities due to their competitive effects with plant pathogens (Hefte et al., 1994).

All the Azospirillum isolates produced both catechol and salicylate type of siderophores. The catechol type of siderophore produced by Azospirillum isolates ranged from 1.60 to 4.83 μg ml⁻¹ and salicylate type ranged from 4.21 to 9.05 μg ml⁻¹ of culture broth. The isolate AZ – 5 Vallampadugai location produced the highest quantity of 4.83 and 9.05 μg ml⁻¹ of catechol type and salicylate type of siderophores. The Azospirillum isolate AZ – 17 Ponnanthittu produced 4.70 and 8.81 μg of catechol and salicylate types of siderophores ml⁻¹ respectively. The minimum amount 1.60 and 4.21 μg of catechol and salicylate type of siderophores ml⁻¹ was respectively produced by the isolate AZ – 20 poondiyankuppam.

Production of siderophore by agriculturally beneficial isolates and its role in Fe mobilization was reported by several workers (Saxena et al., 1986; Kumar and Dube, 1993; Karthikeyan, 1999; Sharma and Johri, 2003) of the two species of Azospirillum, Azospirillum lipoferum produced higher amount of siderophore than Azospirillum brasilense. It was reported earlier by Sridhar and Balasubramanian (1996) that Azospirillum lipoferum secreted higher quantities of siderophore than other Azospirillum species. The finding of the present research was also similar with the findings of above given authors. Based on the screening process of plant growth promoting traits such as ARA activity, cell nitrogen content, GA₃ production, IAA production and Siderophore production by different Azospirillum isolates such as AZ – 5, AZ – 8, AZ – 10, AZ – 11, AZ – 15 and AZ - 17 were selected for interstrain difference studies under saline conditions.

The Arbuscular Mycorrhizal (AM) fungi, a unique group of soil fungi forming symbiotic association with higher plants, facilitate uptake and translocation of phosphorus from soil beyond the root zone of absorption
through proliferation of its hyphae. The capacity of external hyphae for uptake and transport of nutrients viz., nitrogen, phosphorus and potassium has been well demonstrated. AM fungi have great potential to enhance the nutrient uptake, particularly more efficient in phosphorus uptake and plant growth (Sitaramiah et al., 1997).

The results of root colonization percentage and AM fungal spore population was studied in the present research. The tomato root colonization per cent and AM fungal spore population were in the range from 29.50 per cent to 61.32 per cent and 49.00 to 98.00 respectively. The sample collected from Pudhuchattiram recorded the highest root colonization percentage and spores (61.32 per cent and 98.00). The sample collected from Poondiyankuppam recorded the least root colonization percentage and spores (29.50 per cent and 49.00). These results are in agreement with the findings of Miranda and Harris (1994) in Leek and Chandrashekhara et al. (1995) in sunflower.

The AM fungal spore diversity was studied from the tomato rhizosphere soils. The spores of different AM fungal species viz., *Glomus fasciculatum*, *Glomus mosseae*, *Gigaspora margarita* and *Acaulospora laevis* were accounted separately. In general, *Glomus fasciculatum* was the most predominant species followed by *Glomus mosseae*, *Gigaspora margarita* and *Acaulospora laevis*. The number of spores per 100 g⁻¹ soil ranged from 18.00 to 58.00 for *Glomus fasciculatum*, from 10 to 19 for *Glomus mosseae*, from 9.00 to 13.00 for *Gigaspora margarita* and from 5.00 to 13.00 for *Acaulospora laevis*. Among the four isolates, *Glomus fasciculatum* (58 per 100 g⁻¹ soil) recorded the highest AM fungal spore numbers at Pudhuchattiram.

The native AM fungal spores and AM fungal propagule numbers are often correlated with the vegetation, vegetation cover and infective
AM fungal propagules has been demonstrated (Michelsen and Rosendahl, 1988). Correlation between the organic matter content and the AM fungal propagule density was found (Sieverding and Toro, 1986). It is well known that organic matter content of tropical soil decreases with depth. Mycorrhizal colonization of roots was moderate to heavy through the soil profile. The proportion of the root colonized declined moderately with depth of the soil. A rapid decline in colonization by AM fungi with depth is usually observed (Abbott and Robson, 1991).

The AM fungus greatly differs in effectiveness. The effectiveness could be studied either by estimating biomass production, yield or nutrient mobilization. The results of the pot culture study revealed that among four identified AM fungal species tested viz., *Glomus fasciculatum*, *Glomus mosseae*, *Gigaspora margarita* and *Acaulospora laevis* were screened for their efficiency by determining the root infectivity, AM fungal spore number, acid and alkaline phosphatase activity in soil. All the four AM fungal species colonized the roots of maize. However, the degree of root infection and colonization varied considerably between them. The response of maize in terms of root colonization by AM fungi was the highest with *Glomus fasciculatum* followed by *G. mosseae*, *Gigaspora margarita* and *Acaulospora laevis* in soils. Acid and alkaline phosphatase activities were also the highest in *Glomus fasciculatum*. The results of this study were in agreement with Nehi et al. (1999).

The interstrain difference of efficient *Azospirillum* isolates *viz.*, AZ - 5, AZ - 8, AZ -10, AZ - 11, AZ – 15 and AZ - 17 on a generation time (GT) was studied both under N - free and N -supplemented conditions and at different salinity levels *viz.*, 0.1 M, 0.3 M, 0.5 M and 0.8 M NaCl. It was also observed that the increasing levels of salinity increase the generation time of all the six isolates. The *Azospirillum* isolates AZ - 8 recorded a very
low generation time viz., 2.8 hrs and 2.8 hrs at salinity level, respectively in N-free medium whereas it was 1.8 and 1.6 hrs in N-supplemented medium. The results of the present study clearly revealed the positive influence of ‘N’ nutrition and salinity level in determining the generation time of *Azospirillum* isolates. Interstrain differences in *Azospirillum* isolates were studied by many workers (Gaskins *et al*., 1977; Neyra *et al*., 1977; Tarrand *et al*., 1978; Dobereiner and Baldani, 1979; Charyulu and Rao, 1980; Rinaudo *et al*., 1981; Tien *et al*., 1981; Bashan *et al*., 1985; Hurek *et al*., 1985; 1988; Kipe-Nolt *et al*., 1985; Barbieri *et al*., 1988; Favilli *et al*., 1984; 1988; Markus and Kramer, 1988).

The interstrain difference of efficient *Azospirillum* isolates viz., AZ - 5, AZ - 8, AZ – 10, AZ - 11, AZ – 15 and AZ - 17 on nitrogen fixation was studied both under free living condition and in association with tomato plants at 0.3 M NaCl concentration. The isolate AZ – 8 (*A. lipoferum*) from Pudhuchattiram, fixed more nitrogen of 194.2 mole C<sub>2</sub>H<sub>4</sub>/mg/hrs and 250.6 mole/plant hr under free living condition and in association with tomato plant, respectively. Whereas, the *Azospirillum* isolate, AZ - 15, recorded less amount of ‘N’ fixed (136.0 mole C<sub>2</sub>H<sub>4</sub> mg/h and 195.3 mole/plant/h under free living condition and in association with tomato plant, respectively).

Becking (1985) reported the generation time of *Azospirillum brasilense* Kul-9 on yeast extract malate medium as 1.6 hr and in N-free medium as 7 days. Reinhold *et al* (1988) reported the generation time (GT) of *Azospirillum brasilense* Sp7 as 1.6 hr on SM media supplemented with 0.25 per cent NaCl concentration. They also showed the interstrain difference of *Azospirillum* strains in generation time at various levels of NaCl concentration. Hartmann (1988) reported that the generation time of *Azospirillum brasilense* Sp7 as 3 hr in nutrient broth at 0.3 M NaCl concentration.
The interstrain difference of six efficient *Azospirillum* isolates on Indole acetic acid production (IAA) at 0.3 M NaCl concentration was tested. Among the different isolates, *A. lipoferum* (AZ - 8) obtained from tomato rhizosphere of Puduchattirram location was found to produce a higher amount of IAA with 95.50 µg 25 ml⁻¹ of broth at 0.3 M NaCl concentration when compared to other isolates. It was followed by the isolate AZ – 17 ponnanthittu location with 91.20 µg 25 ml⁻¹ of broth and the minimum IAA production was noticed by the isolate AZ – 15 (82.45 µg 25 ml⁻¹ of broth). Variation in the production of IAA by different isolates of *Azospirillum* was reported by Tien *et al* (1979), Francesco *et al* (1987) and Crozier *et al* (1988).

The interstrain difference of *Azospirillum* isolates on siderophore production at 0.3 M NaCl concentration was tested. It was found that all the *Azospirillum* isolates were able to produce siderophore in Nfb broth at 0.3 M NaCl level but with a variation among the isolates. Among the different *Azospirillum* isolates, *Azospirillum* (AZ - 8) was found to produce a higher amount of siderophores (2, 3 - DHBA – 5.15 µg ml⁻¹; salicylic acid – 9.30 µg ml⁻¹) at 0.3 M NaCl concentration when compared to other isolates. Sekar (1995) reported the interstrain difference *Azospirillum* isolates for siderophore production. However, this report showed the interstrain difference of *Azospirillum* isolates, on the production of siderophores under saline stress condition.

The interstrain difference of *Azospirillum* isolates on adhesion of tomato roots at 0.3 M NaCl concentration was estimated. It was found that all the *Azospirillum* isolates were able to adhere tomato roots at 0.3 M NaCl level but with a variation among the isolates. Among the different *Azospirillum* isolates, *Azospirillum* (AZ - 8) was found to exhibit more adherence to tomato roots (178.4 × 10⁴/g dry weight of root/hour) at 0.3 M
NaCl concentration when compared to other isolates. Sukiman and New (1990) and Levanony and Bashan (1991) reported that interstrain differences between *Azospirillum* isolates for adhesion to plant roots. Fischer et al (1999) studied the attachment of *Azospirillum brasilense* to maize and wheat roots and reported that there was an alteration in the adsorption phase of attachment when the bacteria were grown under saline stress.

The interstrain difference of *Azospirillum* isolates on exopolysaccharides production at 0.3 M NaCl concentration was studied. All the *Azospirillum* isolates were able to produce water soluble and alkali soluble exopolysaccharides at 0.3 M NaCl level but with a variation among the isolates. Among the different *Azospirillum* isolates, *Azospirillum* (AZ - 8) was found to produce a higher amount of exopolysaccharides production (Water soluble polysaccharides – 0.068 g per 100 ml; Alkali soluble polysaccharides – 5.726 glucose equivalent/g cell dry weight) at 0.3 M NaCl concentration when compared to other isolates. Hamdia and El–Komy (1997) reported the elevation of soluble polysaccharides level with *Azospirillum* grown under salt stress condition. Haggag (2007), Liu et al (2009) and Liu et al (2010), reported the EPS production by the isolates of *P. polymyxa*.

The desiccation resistance of the *Azospirillum* isolates were studied. Among the *Azospirillum* isolates, AZ-8 was found to have more desiccation resistance when compared to the other isolates. The isolate *Azospirillum* AZ-8 recorded the maximum number of viable cells (7.42 x 10⁴ cells ml⁻¹) after 15 days incubation. Six *Azospirillum* isolates were found to exhibit the thermal resistance at 50°C. Among the six isolates tested, the isolate *Azospirillum* AZ-8 was found to be the best in yielding higher number of viable cells (7.49 x10⁴ cells ml⁻¹) after treatment at 50°C. Sekar (1995) reported the existing of interstrain
difference of Azospirillum isolates with regards to thermal and desiccation tolerance. In the present study, the isolate AZ-8 (Azospirillum lipoferum) exhibited the highest thermal tolerance at 50°C temperature and desiccation tolerance for one week in the incubator and the results of the present study are in conformity with the earlier findings of Sekar (1995).

Tomato (Lycopersicon esculentum L.) is the most widely grown vegetable in the world being recognized as a reach source of vitamins and minerals. It is also among the most important vegetable crops. The total production of this crop in the country has shown a marked increase (Lemma et al., 1992) since it became the most profitable crop providing a higher income to small scale farmers compared to other vegetable crops.

The effect of A. lipoferum (AZ-8) and G. fasciculatum on germination percentage and vigour index in tomato PKM-1 were determined. The maximum germination percentage (96.55 per cent) and vigour index (1737.90) was observed in the treatment T₆ (75 per cent N and P + 100 per cent K + A. lipoferum + G. fasciculatum). The treatment T₆ was found to be statistically significant over the treatment T₂ (100 per cent NPK) with 90.24 per cent and 1729.33 as respective values. Minimum germination percentage (65.68 per cent) and vigour index (896.53) was observed in the treatment T₁ (Control). However, among the individual inoculation of A. lipoferum (AZ–8) favour 88.15 per cent (Germination percentage) and 1428.03 (Vigour index) than Glomus fasciculatum which recorded 82.00 per cent (Germination percentage) and 1243.30 (Vigour index) with 75 per cent N and 100 K inorganic fertilizers.

Hemavathi et al. (2006) reported the increase in plant height, number of branches, fresh weight and P uptake in Ocimum basilicum on inoculation with Glomus fasciculatum + Pseudomonas fluorescens + Bacillus megatherium. Triple inoculation of AMF + Pseudomonas fluorescens + Rhizoctonia solani
the Ocimum plants recorded higher yield as compared to AMF + Rhizoctonia solani (Neeraj and Singh, 2009).

The effect of A. lipoferum (AZ-8) and G. fasciculatum on plant height of tomato (PKM-1) on 30, 60 and 90 DAS were recorded. The control treatment (T$_1$) recorded poor plant height of 40.83 cm, 60.37 cm and 69.83 cm on 30, 60 and 90 DAS respective. The treatment was followed by T$_3$ (75 per cent N and P + 100 per cent K) with 49.50, 75.03 and 84.93 cm as respective values. When the treatment was supplemented with A. lipoferum (AZ-8) isolate (T$_1$), a moderate increase of 52.67 cm, 80.37 cm and 89.90 cm against 50.47 cm, 78.83 cm and 86.70 cm with G. fasciculatum inoculation (T$_3$). The treatment T$_2$ (100 per cent RDF) recorded 52.67 cm, 80.37 cm and 89.80 cm of plant height on 30, 60 and 90 DAS respectively. Significant increase in plant height was observed in the T$_6$ (75 per cent N and P + 100 per cent K + A. lipoferum + G. fasciculatum) treatment with 56.40 cm, 82.43 cm and 94.43 cm at 30, 60 and 90 DAS respectively which are found to be 9.45 per cent, 10.79 per cent and 8.32 per cent increase over the T$_2$ (100 per cent RDF) treatment. Similar findings were reported by Prabhu et al. (2003) and Karpagam et al. (2004) in brinjal.

Hajer et al. (2006) have also reported the reduction in plant height, fresh and dry vegetative biomass in three tomato cultivars grown under sea water salinity. Amini and Ehsanpour (2006) have reported the reduction in vegetative growth of tomato with increasing salinity. Rahman et al. (2006) reported the increase in plant height of tomato mulched with rice straw while lowest height was observed in control under saline soil.

The dry matter production of tomato (PKM-1) was influenced by different treatments. The minimum dry matter production of 6.89 g/plant was recorded in control treatment. Whereas, the 100 per cent RDF (T$_2$) recorded 10.23 g/plant. When the inorganic fertilizers N and P reduced and
25 per cent (T₃ – 75 per cent N and P + 100 per cent K) reduction of 0.20 g/plant on dry matter production was observed. A marked increase on dry matter production were observed in the treatments viz., T₄ (10.23 g/plant) and T₅ (10.08 g/plant) which were received A. lipoferum (AZ-8) and G. fasciculatum along with 75 per cent N and P + 100 per cent K inorganic fertilizer. The maximum dry matter production of 3.05 g/plant, 7.25 g/plant and 11.98 g/plant on 30 DAS, 60 DAS and 90 DAS were observed in T₆ (75 per cent N and P + 100 per cent K + A. lipoferum + G. fasciculatum) treatment. Similar response in tomato was reported by Palaniappan et al. (1999).

Adams (1988) also reported that growth of tomato plants was stimulated by increasing salinity to 4 to 5 mS/cm due to application of sodium chloride. However, many investigators observed a significant decline in dry matter yield of tomato plants by increasing salinity. Variations in dry matter production were also dependent on salt types and among the soluble salts. NaCl is the most detrimental to plant growth and nutrient uptake (Al-Rawahy et al., 1990).

There were similarities in these results and those of Hernandez and Chailloux (2004), who reported that the dry weight of tomato transplants grown in the greenhouse with 75 per cent fertilizer plus two co-inoculated PGPR was significantly greater than those with full fertilizer rate without PGPR.

Yang et al. (2006) have reported the increased dry matter accumulation with application of mulch in wheat under salinity. Aseri et al. (2008) reported significant interaction of Azotobacter chroococcum and Glomus mosseae in pomegranate leading to better leaf area, shoot dry weight, and uptake of N, P, and K compared to either PGPR or AMF alone.
The effect of *A. lipoferum* (AZ-8) and *G. fasciculatum* on average number of branches plant\(^{-1}\) were counted and the observations were recorded on 30\(^{th}\) day, 60\(^{th}\) day and 90\(^{th}\) day. Inoculation of *A. lipoferum* (AZ-8) and *G. fasciculatum* significantly increased the number of branches per plant than other treatments. It recorded 13.45 branches per plant against 12.33 branches per plant (T\(_2\) – 100 per cent RDF). The results clearly revealed that *A. lipoferum* (AZ-8) and *G. fasciculatum* moderately increased as 12.33 (T\(_3\) – 75 per cent N and P + 100 per cent K) on 90 DAS. The lowest number of branches 9.33 per plant was observed in T\(_1\) (Control) treatment.

An earlier study conducted by Raghu *et al.* (2005) supports these findings where the highest plant biomass (Shoot and root) was observed in *Dalbergia sissoo* an inoculation with *Glomus fasciculatum* + *Azotobacter chroococcum* + *Bacillus coagulans* + *Trichoderma harzianum*. Similarly, Divyananda *et al.* (2005) reported the maximum plant biomass in *Acacia auracilformin* on combined inoculation of *Scutellospora calospora*, *Bacillus coagulans*, *Trichoderma harzianum* and *Azotobacter*, Similarly, Muthuraj and Jayashella (2005) recorded the highest shoot and root dry weight in *Capsicum annum* on treatment with *Pseudomonas fluorescent* + *Glomus mosseae* + *Azospirillum brasilense* compared to other treatments.

Zaidi and Khan (2006) found increased dry matter yield in green gram plants on treatment with triple inoculation of *Glomus fasciculatum*, *Bradyrhizobium* and *Bacillus subtilis*. Increasing in the shoot and root dry weight in our study may be due to synergistic or additive effects of combined inoculation, resulting in a favorable plant AMF- microbial interaction.

The effect of *A. lipoferum* (AZ-8) and *G. fasciculatum* on number of fruits, fruit weight and fruit yield in tomato (PKM-1) were studied. The maximum number of fruits (45.08 plant\(^{-1}\)), fruit weight (65.15 g plant\(^{-1}\)) and
fruit yield (24.05 t ha\(^{-1}\)) was recorded in the treatment T\(_6\) (75 per cent N and P + 100 per cent K + \textit{A. lipoferum} + \textit{G. fasciculatum}). It was followed by T\(_4\) with 41.44 fruits per plant, 61.20 g per fruit and 17.45 t ha\(^{-1}\), T\(_3\) (75 per cent N and P + 100 per cent K) with 36.30 fruits per plant, 58.40 g per fruit and 16.90 t ha\(^{-1}\) and T\(_2\) (100 per cent RDF) with 43.43 fruits per plant, 62.78 g per fruit and 19.25 t ha\(^{-1}\) as respective values. The minimum number of fruits (31.25 plant\(^{-1}\)), fruit weight (49.64 g plant\(^{-1}\)) and fruit yield (9.10 t ha\(^{-1}\)) was observed in the treatment T\(_1\) (Control). These findings are in conformity with the results of Nanthakumar and Veeraragavathatham (1999) and Narayanamma \textit{et al.} (2006) in brinjal.

Bagyaraj and Menge (1978) reported in the per cent root colonization and spore count might be due to the positive interaction effects by \textit{Glomus mosseae} with \textit{Azotobacter chroococcum} \textit{Pseudomonas fluorescens} + \textit{Azospirillum awamari} in the root zone of \textit{Ocimum sanctum}.

According to Srihari (1997), among several species of AM fungi \textit{Glomus fasciculatum} was found effective in the nursery and was able to save upto 25 per cent of phosphatic fertilizer both in red and black soil. While, Vasanth Kumar (2003), screened several endophytic \textit{Azospirillum} sp., identified \textit{Azospirillum} sp. strain Azo V6 capable of fixing atmospheric nitrogen to an extent of 19.26 mg per g of malate. This inoculation was found to benefit tomato in increasing the fruit yield as seed inoculation in pot culture.

Mehla \textit{et al.} (2000) and Pandey \textit{et al.} (1996) reported that fruit yield of tomato is highly influenced by the N and P fertilizers rates applied. Similarly, Sharma \textit{et al.} (1999) also reported that average fruit weight of tomato have been influenced by the amount of N and P fertilizers rates applied. Thus, tomato plant should receive optimum amount of N and P fertilizers to produce higher fruit yields.
Muthuraj et al. (2005) reported higher plant biomass in *Lycopersican esculentum* on treatment with combined inoculation of *Glomus moseae*, *Pseudomonas* compared to other treatments. Charon et al. (2007) also found an increase in the secondary roots on inoculation of tobacco seedlings with *T. harzianum*. Recently, Tanwar et al. (2010) reported the levels in triple combinations of *G. mosseae* + *A. laevis* + *T. harzianum* were tested for their ability to increase yield, biomass and establishment of the tomato (*Lycopersican esculentum* mill) seedlings in pot cultures.

Nitrogen is a necessary component which is used for the growth of the plant. Plants require a limited amount of nitrogen for their growth. The types of crops also determine the level of nitrogen. Some crops require more nitrogen for their growth while, some crops require less amount of nitrogen. Biological nitrogen fixation is estimated to contribute $180 \times 10^6$ metric tons/year globally of which 80 per cent comes from symbiotic associations and the rest from free-living or associative systems. This includes symbiotic nitrogen fixing forms namely: *Rhizobium*, the obligate symbionts in leguminous plants, *Frankia* in non-leguminous trees and non-symbiotic N$_2$ - fixing forms such as Cyanobacteria, *Azospirillum*, *Azotobacter*, *Acetobacter diazotrophicus*, and *Azoarcus*, etc. (Tilak et al., 2005).

The inoculation effect of *A. lipoferum* (AZ-8) and *G. fasciculatum* on the available nutrients (N, P and K) status were estimated. The treatment T$_6$ (75 per cent N and P + 100 per cent K + *A. lipoferum* + *G. fasciculatum*) (N - 197.06 kg ha$^{-1}$; P - 45.97 kg ha$^{-1}$; K - 162.38 kg ha$^{-1}$) showed the maximum available nutrients than other treatments. This treatment was followed by T$_4$ (75 per cent N and P + 100 per cent K + *A. lipoferum*) with available nitrogen kg ha$^{-1}$ (192.37), available phosphorus kg ha$^{-1}$ (42.30) and available potassium kg ha$^{-1}$ (155.62), T$_3$ (75 per cent N and P + 100 per cent K) available nitrogen kg ha$^{-1}$ (179.95), available phosphorus kg ha$^{-1}$
(35.89), available potassium kg ha\(^{-1}\) (140.21) and \(T_2\) (100 per cent RDF) with available nitrogen kg ha\(^{-1}\) (193.83), available phosphorus kg ha\(^{-1}\) (41.37) and available potassium kg ha\(^{-1}\) (158.67) as respective values. The control (\(T_1\)) recorded the lowest available nutrients (\(N = 161.34\) kg ha\(^{-1}\); \(P = 21.69\) kg ha\(^{-1}\); \(K = 128.04\) kg ha\(^{-1}\)). This positive effect on plant growth is in agreement with the studies of Gnekow and Marschner (1989), in which mycorrhizal growth-enhancement of apple remained significant in substrates containing high levels of extractable P.

The inoculation effect of \(A.\) lipoferum (AZ-8) and \(G.\) fasciculatum on the available nutrients (N, P and K) status in tomato plant were estimated. The treatment \(T_6\) (75 per cent \(N\) and \(P + 100\) per cent \(K + A.\) lipoferum + \(G.\) fasciculatum) (\(N = 196.17\) kg ha\(^{-1}\); \(P = 45.08\) kg ha\(^{-1}\); \(K = 161.49\) kg ha\(^{-1}\)) showed the maximum available nutrients than other treatments. The control (\(T_1\)) recorded the lowest available nutrients (\(N = 178.45\) kg ha\(^{-1}\); \(P = 37.24\) kg ha\(^{-1}\); \(K = 135.90\) kg ha\(^{-1}\)).

The bacterial strains had much better effect on the growth and nutrient uptake of plants in nutrient-deficient saline serozem soil than in relatively rich loamy sand.

According to Paula \textit{et al.} (1992), the magnitude of the plant response to any microbial inoculation can be greatly affected by the soil condition. Inoculation of plants with bacteria only marginally increased yields when tested under ideal climatic situations. The greatest benefits occurred when crops encountered stressful conditions (Lazarovits and Norwak, 1997), while, nontreated plants by comparison performed poorly under such conditions where high pH make nutrients less available to them.

The inoculation of \(A.\) lipoferum and \(G.\) fasciculatum significantly increased the nutrient uptake (N, P and K). The treatment \(T_6\) (75 per cent \(N\) and \(P + 100\) per cent \(K + A.\) lipoferum + \(G.\) fasciculatum) (\(N = 2087.72\) kg ha\(^{-1}\),
P-451.55 kg ha\(^{-1}\); K - 1690.75 kg ha\(^{-1}\)) showed the maximum uptake of nutrients when compared to other treatments. The treatment T\(_1\) recorded the lowest nutrient uptake (N - 1111.63 kg ha\(^{-1}\); P - 149.44 kg ha\(^{-1}\); K - 882.19 kg ha\(^{-1}\)).

Similar results were reported by Defreitas and Germida (1992) that in low fertility soil, *Pseudomonas* significantly enhanced early plant growth. Such inoculation could compensate for nutrient deficiency and improve plant development through the production of plant growth regulators by microbes at the root interface, which stimulated root development and resulted in better absorption of water and nutrients from the soil (Wu *et al.*, 2005). PGPR may enhance mineral uptake including N, P, K, and microelements more efficiently from the soil, not only as a consequence of the increase in root surface area, but also by stimulating the ion uptake systems (Burdman *et al.*, 2000).

Defreitas and Germida (1992) showed that several PGPB strains increased root hair size and number, and these tubular extensions of root epidermal cells can be involved in mineral uptake capacity in two ways. First, root hairs represent a large surface available for ion uptake and, second, they are believed to play an important role in nutrient uptake.

Earlier reports claim that soil salinity has an adverse effect on plant growth-promoting bacterial populations, but members of potentially pathogenic species survive and become enriched in the rhizosphere (Sato and Jiang, 1996; Tripathi *et al.*, 2002).

The effect of *A. lipoferum* (AZ-8) and *G. fasciculatum* inoculation in tomato (PKM-1) on the microbial population was studied. The highest microbial population (Bacteria - \(56.85 \times 10^6\) CFU g\(^{-1}\); Fungi - \(1.55 \times 10^5\) CFU g\(^{-1}\) and Actinobacteria - \(15.37 \times 10^4\) CFU g\(^{-1}\)) was recorded in the treatment T\(_6\) (75 per cent N and P + 100 per cent K + *A. lipoferum* +
G. fasciculatum). The treatment T₇ was on par with the treatment T₂ (100 per cent NPK) (Bacteria – \(48.72 \times 10^6\) CFU g⁻¹; Fungi – \(1.52 \times 10^5\) CFU g⁻¹ and Actinobacteria – \(14.30 \times 10^4\) CFU g⁻¹). Lowest microbial population (Bacteria – \(28.58 \times 10^6\) CFU g⁻¹; Fungi – \(1.30 \times 10^5\) CFU g⁻¹ and Actinobacteria– \(9.44 \times 10^4\) CFU g⁻¹) was observed in the treatment T₁ (Control).

Inoculation of A. lipoferum (AZ-8) and G. fasciculatum in tomato (PKM-1) significantly influenced on population of Azospirillum sp. and AM fungal spores. The least Azospirillum population \(0.14 \times 10^6\) CFU g⁻¹ of soil and AM fungal spore population \(13.80/100\) g rhizosphere soil was recorded in the control (T₁) treatment. The corresponding values for T₂ (100 per cent RDF) were \(7.75 \times 10^6\) CFU g⁻¹ and \(23.12/100\) g soil and T₃ (75 per cent N and P + 100 per cent K) were \(4.85 \times 10^6\) CFU g⁻¹ and \(15.65/100\) g soil were observed.

Whereas, the individual and dual inoculated treatments (T₄, T₅ and T₆) significantly increased the population over control and inorganic fertilizer applications. Among these three limits, the maximum population of \(9.85 \times 10^6\) CFU g⁻¹ of soil (Azospirillum sp.) and \(45.25\) spores/100 g soil (AM fungal spores population) were observed. The respective values for T₄ (75 per cent N and P + 100 per cent K + A. lipoferum) were \(5.50 \times 10^6\) CFU g⁻¹ and \(20.18/100\) g soil, and T₅ (75 per cent N and P + 100 per cent K + G. fasciculatum) were \(1.75 \times 10^6\) CFU g⁻¹ and \(20.75\) spores/100 g of soil.

Klyuchnikov and Kozhevin (1990) studied the population dynamics of Azospirillum brasilense during the growth cycle of potato as influenced by G. mosseae and they recorded the most numerous and active populations of A. brasilense in the rhizosphere and endorhizosphere.

Muthuraj and Jayasheela (2005) recorded significantly higher per cent root colonization and spore count in the rhizosphere of Capsicum
annum on the combined inoculation with *Glomus mosseae* + *Pseudomonas fluorescens* + *Azospirillum brasilense*.

The effect of *A. lipoferum* (AZ-8) and *G. fasciculatum* on the chlorophyll content in tomato leaf was estimated in the current study. The maximum chlorophyll content (2.78 mg g⁻¹) was recorded in the treatment T₆ (75 per cent N and P + 100 per cent K + *A. lipoferum* + *G. fasciculatum*) on 90th day. It was followed by T₄ (75 per cent N and P + 100 per cent K + *A. lipoferum*) and T₅ (75 per cent N and P + 100 per cent K + *G. fasciculatum*). Whereas, the 100 per cent RDF (T₂) recorded 2.53 mg g⁻¹ and the minimum content of 1.70 mg g⁻¹ was observed in the control (T₁) treatment.

Amini and Ehsanpour (2006) reported the decrease in chlorophyll content in tomato cultivar due to salt stress. Khavari and Mostofi (1998) found reduction in photosynthetic pigments with increasing salinity level in tomato cultivars. Yang et al. (2006) have reported increased chlorophyll content and dry matter accumulation with application of mulch in wheat under salinity.

The carbohydrate content of tomato (PKM-1) fruits as influenced by different treatments. The minimum carbohydrate content of 1.95 mg/g was observed in control (T₁). It was followed by T₃ (75 per cent N and P + 100 per cent K) with 2.07 mg/g and T₂ on 2.28 mg/g on 90th day. When the plants were inoculated with *A. lipoferum* (AZ-8) and *G. fasciculatum* (T₄ – 75 per cent N and P + 100 per cent K + *A. lipoferum* and T₅ – 75 per cent N and P + 100 per cent K + *G. fasciculatum*) as individual and dual (T₆ – 75 per cent N and P + 100 per cent K + *A. lipoferum* + *G. fasciculatum*) increase in carbohydrate content were observed. Among the individual inoculation treatments, T₄ (75 per cent N and P + 100 per cent K + *A. lipoferum*) recorded more carbohydrate content as 2.08, 2.12 and 2.24 mg/g and
$T_3$ (75 per cent N and P + 100 per cent $K + G. fasciculatum$) recorded 2.08, 2.13 and 2.25 mg/g on 30, 60 and 90 DAS respectively. The highest carbohydrate content of 2.23, 2.35 and 2.48 mg/g on 30, 60 and 90 DAS respectively were recorded in $T_6$ (75 per cent N and P + 100 per cent $K + A. lipoferum + G. fasciculatum$). Lopez-Berenguer et al. (2004) observed increase in soluble carbohydrate in pepper under saline irrigation. Adams (1991) and Mirzahi et al. (1988) found similar results in tomato.

Similar to carbohydrate content, the maximum lipid content of tomato (PKM-1) was also recorded. Among the treatments, the treatment $T_6$ (75 per cent N and P + 100 per cent $K + A. lipoferum + G. fasciculatum$) with 1.86 mg/g followed by $T_4$ (75 per cent N and P + 100 per cent $K + A. lipoferum$) with 1.82 mg/g, $T_5$ (75 per cent N and P + 100 per cent $K + G. fasciculatum$) with 1.67 mg/g. the 100 per cent RDF ($T_2$) recorded 1.75 mg/g, $T_3$ recorded 1.55 mg/g and the minimum content of 1.30 was found in control ($T_1$).

The effect of $A. lipoferum$ (AZ – 8) and $G. fasciculatum$ on total protein content of tomato fruits was estimated. Significant increase in the protein content was observed in the treatments received bioinoculants ($T_4 – 75$ per cent N and P + 100 per cent $K + Azospirillum lipoferum$, $T_5 – 75$ per cent N and P + 100 per cent $K + Glomus fasciculatum$ and $T_6 – 75$ per cent N and P + 100 per cent $K + A. lipoferum + G. fasciculatum$) over inorganics ($T_2 – 100$ per cent RDF and $T_3 – 75$ per cent N and P + 100 per cent K) and control ($T_1$). Among the six treatments, $T_6$ (75 per cent N and P + 100 per cent $K + A. lipoferum + G. fasciculatum$) recorded the maximum of 0.62 mg/g followed by $T_4$ (75 per cent N and P + 100 per cent $K + Azospirillum lipoferum$) with 0.60 mg/g and $T_5$ (75 per cent N and P + 100 per cent $K + Glomus fasciculatum$) with 0.53 mg/g. Kotoky and Bhattacharyya (1992) found the highest protein content in leaves of banana
associated with application of organic mulch. Areghere and Tofinga (2004) obtained higher amount of crude proteins in sweet potato under the various types of organic mulches.

Similar to other biochemical constituents, fibre content of tomato was also enhanced by the inoculation of \textit{A. lipoferum (AZ-8)} and \textit{G. fasciculatum} as individual and dual inoculations over inorganics and control. The lowest fibre content of 1.57 mg/g was reported in \textit{T}_1 (Control). Among the bioinoculant treatments, the maximum fibre content was recorded in \textit{T}_6 (1.85 mg/g). This result was supported by the findings of Adams (1991).

The effect of \textit{A. lipoferum (AZ-8)} and \textit{G. fasciculatum} on ascorbic acid content was studied. The maximum ascorbic acid content (33.85 mg g\(^{-1}\)) was recorded in the treatment \textit{T}_6 (75 per cent N and P + 100 per cent K + \textit{A. lipoferum + G. fasciculatum}). The lowest ascorbic acid content (21.15 mg g\(^{-1}\)) was observed in the treatment \textit{T}_1 (Control). These findings were supported by Lopz-Berenguer \textit{et al.} (2004).

The inoculation effect of \textit{A. lipoferum (AZ-8)} and \textit{G. fasciculatum} on carotenoids content was tested. The maximum carotenoids content (\textit{All trans lutein} – 18.95 mg g\(^{-1}\); \textit{β – carotene} – 78.88 mg g\(^{-1}\); 9 – \textit{Cis β – carotene} – 12.30 mg g\(^{-1}\); lycopene – 360 mg g\(^{-1}\); lycopene isomers – 130 mg g\(^{-1}\) and neurosporene – 33.12 mg g\(^{-1}\)) was recorded in the treatment \textit{T}_6 (75 per cent N and P + 100 per cent K + \textit{A. lipoferum + G. fasciculatum}). The lowest carotenoids content (\textit{All trans lutein} – 14.20 mg g\(^{-1}\); \textit{β – carotene} – 66.60 mg g\(^{-1}\); 9 – \textit{Cis β – carotene} – 10.95 mg g\(^{-1}\); lycopene – 325 mg g\(^{-1}\); lycopene isomers – 102 mg g\(^{-1}\) and neurosporene – 21.90 mg g\(^{-1}\)) was observed in the treatment \textit{T}_1 (Control). The results were in similar with the findings of Areghere and Tofinga (2004).